> (e.g., exons, domain), encompassed by the claimed invention. Although the claims have been amended to provide more structure and function; the scope of the claims is not commensurate with the enablement provided by the disclosure with regard to the ill-defined number of known and unknown nucleic acids broadly encompassed by the claims." See, Office Action page 3 , lines 14-19.

This rejection is respectfully traversed.

It is Applicants' position that claims 1-17, 30-31, 33-47, 60-61, 63-65, 69-71 and 75-77 are definite in that they are all directed to novel nucleic acid molecules which are naturally occurring variants of the nucleotide sequence shown in SEQ ID NO: 18 or SEQ ID NO:22. The instant costimulatory molecules are alternative splice forms of the prior art B7-1 and/or B7-2 molecules. It is Applicants' position that the nucleic acid sequences and exon structure of the prior art non-alternatively spliced B7-1 and B7-2 molecules are taught by Applicants in the instant specification and were well known in the art at the time of filing the application. Applicants further contend that the protein domains of the B7-1 and B7-2 molecules were also taught in the specification (see, e.g., page 16 of the application where the amino acid positions of certain domains are set forth) and were known in the art.

Since the claims as pending are drawn to naturally occurring variants of B7-1 and B7-2 molecules and refer to exons and domains of B7-1 and B7-2 molecules which were well known in the art, it is Applicants' position that the claims provide sufficient structural information and defining characteristics which would be readily understood by one of ordinary skill in the art. Moreover, several references which describe in detail the specific structural determents of the B7-1 and B7-2 domains are incorporated by reference in their entirety (as if fully set forth in the present application), further defining and enabling the claimed molecules.

For example, with respect to the immunoglobulin variable region-like domain, as described in Freeman *et al.* (1989) *J. Immunology* 143: 2714-2722; Freeman *et al.* (1991) *J. Exp. Med.* 174: 625-631; Freeman *et al.* (1993) *Science* 262: 909-911; and Selvakumar *et al.* (1993) *Immunogenetics* 38: 292-295, each of which was incorporated by reference

into the specification, this domain comprises an extracellular region of a B7-1 or B7-2 molecule which is similar in structure to art-known immunoglobulin variable regions, and as such includes nine anti-parallel β-strands and an intradomain salt bridge. With respect to the immunoglobulin constant region-like domain, also described in Freeman *et al.* (1989), Freeman *et al.* (1991), Freeman *et al.* (1993), and Selvakumar *et al.* (1993), this domain comprises an extracellular region of a B7-1 or B7-2 molecule which is similar in structure to art-known immunoglobulin constant region domains, and as such is known to have seven antiparallel β-strands.

It is known that the transmembrane domain of a B7-1 or B7-2 molecule is that portion of the protein which anchors the molecule into the cellular membrane. As discussed in Freeman *et al.* (1989), Freeman *et al.* (1991), Freeman *et al.* (1993), and Selvakumar *et al.* (1993), this short domain (e.g., fewer than 30 amino acids) has at least two cysteine residues and is very hydrophobic, thereby able to permit interactions with the hydrophobic core of the membrane lipid bilayer. It is also known that the cytoplasmic domain of a B7-1 or B7-2 molecule is that portion of the protein which is present in the cytosol of the cell. As described in Freeman *et al.* (1989), Freeman *et al.* (1991), Freeman *et al.* (1993), and Selvakumar *et al.* (1993), this domain is highly positively charged.

Signal peptides are molecules also well known in the art which direct the delivery of a molecule to which they are attached to a specific region of the cell (i.e., the nucleus, or the endoplasmic reticulum) or to the extracellular milieu. While a number of different signal peptide sequences directing export of the protein molecule to which they are attached are known in the art, each has a similar art-known structure in the presence of a lipidic environment which contributes to the ability of the sequence to disrupt a membrane and to thereby permit translocation of the polypeptide chain to the extracellular surroundings (for review, see Jones, J.D. et al. (1990) J. Bioenerg. Biomembr. 22 (3): 213-232, a copy of which is enclosed). Thus, it is well understood to those skilled in the art that a "signal peptide domain" as recited in the claims refers to a short (generally less than 40 amino acids) art-known domain with a sequence permitting the formation of an α-helical structure.

Furthermore, one skilled in the art would be aware of amino acid substitutions which would conserve the above-mentioned structural characteristics of the B7-1 or B7-2 domains. For example, both arginine and lysine are positively charged amino acids, and in order to preserve the highly positively charged structure of the cytoplasmic domain, these amino acids may be substituted for one another. Similarly, hydrophobic amino acids are well-known in the art. Further, the ability of a specific set of amino acid residues to form a particular tertiary structure can be calculated by art-known methods. Thus, the ability of a particular sequence of amino acids to form, for example, an antiparallel β-strand, is readily determinable by art-known methods.

In addition to meeting the structural requirements of the claims, the subject nucleic acid molecules must encode a functional B7-1 or B7-2 molecule which binds to CD28 or CTLA4. Thus, the claims only embrace B7-1 and B7-2 nucleic acid molecules which encode functional T cell costimulatory molecules and do not read on inoperative species. Furthermore, Applicants provide sufficient guidance to enable one of ordinary skill in the art to determine whether a given nucleic acid molecule encodes a B7-1 or B7-2 molecule with costimulatory activity. For example, at least at page 30, Applicants teach that the novel T cell costimulatory molecules of the invention can be expressed in membrane bound form by expressing the molecule in a host cell (e.g., by transfecting the host cell with a recombinant expression vector encoding the molecule). Applicants teach that to trigger a costimulatory signal, T cells are contacted with the cell expressing the costimulatory molecule, preferably together with a primary activation signal (e.g., MHC-associated antigenic peptide, anti-CD3 antibody, phorbol ester etc.). Activation of a T cell can be assayed by standard procedures, for example by measuring T cell proliferation or cytokine production. The methods taught by Applicants, as well as other methods of measuring costimulatory signals known in the art, could readily be used to determine whether a nucleic acid molecule functions according to the limitations of the claim without undue experimentation.

It is therefore Applicants' position that, given Applicants' teachings, it would not require undue experimentation to practice the invention in a manner commensurate in scope with the claims, which recite art–known domains having structural and functional limitations as set forth above. For example, beginning at page 14 and again at page 37,

Applicants teach that cDNAs encoding the costimulatory molecule can be amplified by reverse transcriptase-polymerase chain reaction (RT-PCR) using oligonucleotide primers specific for the costimulatory molecule gene. The amplified cDNAs can then be subcloned into a plasmid vector and sequenced by standard methods. Oligonucleotide primers for RT-PCR can be designed based upon previously disclosed nucleotide sequences of costimulatory molecules (see, Freeman, G.J. et al., (1991) *J. Exp. Med.* 174:625-631 for mB7-1; Freeman, G.J. et al., (1989) *J. Immunol.* 143:2714-2722 for hB7-1; Freeman, G.J. et al., (1993) *J. Exp. Med.* 178:2185-2192 for mB7-2; and Freeman, G.J. et al., (1993) *Science* 262:909-911 for hB7-2; nucleotide sequences are shown in SEQ ID NOS: 16, 18, 20, 22 and 24. Also, Table 1, page 38 and page 43). For analyzing the 5' or 3' ends of mRNA transcripts, cDNA can be prepared using a 5' or 3' "RACE" procedure ("rapid amplification of cDNA ends") as described in the Examples. Alternative to amplifying specific cDNAs, a cDNA library can be prepared from a cell line which expresses the costimulatory molecule and screened with a probe containing all or a portion of the nucleotide sequence encoding the costimulatory molecule.

Applicants further teach that individual isolated cDNA clones encoding a T cell costimulatory molecule can then be sequenced by standard techniques, such as dideoxy sequencing or Maxam-Gilbert sequencing, to identify a cDNA clone encoding a T cell costimulatory molecule having a novel structural domain. A novel structural domain can be identified by comparing the sequence of the cDNA clone to the previously disclosed nucleotide sequences encoding T cell costimulatory molecules (e.g., sequences shown in SEQ ID NO: 16, 18, 20, 22 and 24). Once a putative alternative structural domain has been identified, the nucleotide sequence encoding the domain can be mapped in genomic DNA to determine whether the domain is encoded by a novel exon.

Alternatively, Applicants teach that a novel structural domain for T cell costimulatory molecules can be identified in genomic DNA by identifying a novel exon in the gene encoding the T cell costimulatory molecule. A novel exon can be identified as an open reading frame flanked by splice acceptor and splice donor sequences. Genomic clones encoding a T cell costimulatory molecule can be isolated by screening a genomic DNA library with a probe encompassing all or a portion of a nucleotide sequence encoding the costimulatory molecule (e.g., having all or a portion of a

nucleotide sequence shown in SEQ ID NO: 16, 18, 20, 22 and 24). For costimulatory molecules whose genes have been mapped to a particular chromosome, a chromosome-specific library rather than a total genomic DNA library can be used. For example, hB7-1 has been mapped to human chromosome 3 (see, Freeman, G.J. et al. (1992) *Blood* 79:489-494; and Selvakumar, A. et al. (1992) *Immunogenetics* 36:175-181). Genomic clones can be sequenced by conventional techniques and novel exons identified. A probe corresponding to a novel exon can then be used to detect the nucleotide sequence of this exon in mRNA transcripts encoding the costimulatory molecule (e.g., by screening a cDNA library or by PCR).

Applicants further teach that a more preferred approach for identifying and isolating nucleic acid encoding a novel structural domain of a T cell costimulatory molecule is by "exon trapping". Exon trapping is a technique that has been used successfully to identify and isolate novel exons (see, e.g. Duyk, G.M. et al. (1990) *Proc. Natl. Acad. Sci. USA* 87:8995-8999; Auch, D. and Reth, M. (1990) *Nucleic Acids Res.* 18:6743-6744; Hamaguchi, M. et al. (1992) *Proc. Natl. Acad. Sci. USA* 89:9779-9783; and Krizman, D.B and Berget, S.M. (1993) *Nucleic Acids Res.* 21:5198-5202). The approach of exon trapping can be applied to the isolation of exons encoding novel structural domains of T cell costimulatory molecules, such as a novel alternative cytoplasmic domain of human B7-1, as described in Example 5.

Thus, it is Applicants' position that the pending claims are definite and are fully enabled by the specification as filed. Therefore, Applicants respectfully request that the rejection of the claims under 35 U.S.C. § 112, first and second paragraphs be reconsidered and withdrawn.

Applicants acknowledge the withdrawal of the prior art rejections under 35 U.S.C. §§102/103.

In view of the foregoing, Applicants respectfully submit that claims 1-17, 30-31, 33-47, 60-61, 63-65, 69-71 and 75-77 are patentable. If a telephone conversation with Applicants' Attorney would expedite prosecution of the above-identified application, the Examiner is urged to call Applicants' Attorney at the number provided below.

Respectfully submitted,

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